



Effect of elicitors on physiomorphological and biochemical parameters of Indian mustard (*Brassica juncea*) and rapeseed (*B. napus*)

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Abstract: The present investigation was aimed to assess the effect of different concentrations of elicitors viz., salicylic acid (SA) and benzothiadiazole (BTH) on physiomorphological and biochemical parameters of *Brassica juncea* and *B. napus* cultivars. The field experiment was conducted during 2012-13 at Punjab Agricultural University, Ludhiana, India. Four different treatments of elicitors and a fungicide treatment were given to 10 week old plants up to four consecutive weeks. Content of total soluble protein, free amino acids, total sugars and reducing sugars of leaves of both *B. juncea* and *B. napus* were evaluated after each spray. The content of total soluble protein increased, whereas the contents of free amino acids, total sugars and reducing sugars decreased in all the treatments after each spray. Among all the treatments, the combinations of elicitors, i.e., BTH (3 ppm) + SA (33 ppm) and BTH (7 ppm) + SA (17 ppm) exhibited maximum contents of total soluble protein, free amino acids, total sugars and reducing sugars than control in both the cultivars. These treatments were more pronounced in increasing plant height, internodal distance and number of pods per plant in both the cultivars. Our results suggest that the combinations of elicitors act synergistically to promote growth and metabolic activities in *B. juncea* and *B. napus* cultivars leading to the induction and regulation of disease resistance.

Keywords: Benzothiadiazole, *Brassica*, Free amino acids, Salicylic acid, Total soluble protein, Total sugars

INTRODUCTION

Brassica juncea (Indian mustard) and *B. napus* (rapeseed) grouped under rapeseed-mustard, contribute about 32% of the total oilseed production in India. Among the biotic stresses, Alternaria blight caused by *Alternaria brassicae* is the major yield and quality reducing factors of rapeseed-mustard. Depending upon disease severity, about 47% yield loss has been estimated in India (Meena *et al.*, 2010). Resistance to Alternaria blight in rapeseed-mustard has been found to be associated with increased activities of antioxidative enzymes and accumulation of specific metabolites i.e. proteins, free amino acids and sugars (Daayf *et al.*, 2000). Although, fungicides can provide resistance against the disease but these have poor compliances including health hazards. Recently, low molecular weight synthetic molecules called elicitors have been used to alleviate infection by activating plant's own defence system (Mandavia *et al.*, 2012). The use of elicitors lacks environmental and toxicological side-effects and is effective against a variety of plant pathogens. Such elicitors are mainly plant hormones or synthetic analogs.

Salicylic acid (SA) and its chemical analogue benzothiadiazole (BTH) are the most commonly used elicitors. Exogenous application of SA and BTH

activate a number of defense related genes, leading to plant protection against various pathogens (Vernooij *et al.*, 1995; Lawton *et al.*, 1996). These two have successfully been used to induce resistance to a wide range of diseases on field crops (Abdel-Monaim *et al.*, 2011; Farouk and Osman, 2012; Mandavia *et al.*, 2012). They also play an important role in regulating many aspects of plant growth and development (Raskin, 1992).

Plant metabolites like proteins, free amino acids and total sugars are involved in the responses to a number of stresses, and act as nutrient and signaling molecules that activate specific or hormonal-crosstalk transduction pathways, resulting in important modifications of gene expression (Couee *et al.*, 2006). The present study, therefore, was carried out to investigate the effect of elicitors viz., SA and BTH alone or in combinations on physiomorphological and biochemical parameters of *B. juncea* and *B. napus* cultivars.

MATERIALS AND METHODS

Seeds of *B. juncea* (cv. PBR-91) and *B. napus* (cv. GSC-6) were procured from Oilseeds Section, Department of Plant Breeding and Genetics, Punjab Agricultural University (PAU), Ludhiana, India. A field experiment was conducted at the experimental

field of PAU, Ludhiana (30°54'N latitude and 75°48'E longitude) during *rabi* season of 2012-13. The seeds were sown in plant spacing of 30 x 10 cm for cv. PBR-91 and 45 x 10 cm for cv. GSC-6 in plot size of 3 x 4 m² for each treatment.

The treatments comprised of benzothiadiazole (10 ppm), salicylic acid (50 ppm), benzothiadiazole (3 ppm) + salicylic acid (33 ppm), benzothiadiazole (7 ppm) + salicylic acid (17 ppm), fungicide - 0.25% Blitox (50% copper oxychloride) and a control (water-spray). The treatments were replicated three times for both the cultivars. The first foliar spray of elicitors/fungicide was given to 10 week old plants as the first symptom of *Alternaria* blight disease appeared. A total of four foliar sprays of elicitors and three sprays of fungicide (as recommended by agronomic practices) were given on consecutive weeks.

Leaves were sampled after 72 hrs of treatment and used for the estimation of total soluble proteins (Lowry *et al.*, 1951). Half of the leaves were dried at 50-60°C and used for the estimation of total free amino acids (Jayaraman, 1981), total sugars (Dubois *et al.*, 1956) and reducing sugars (Miller, 1972). The plant height, internodal distance and number of pods per plant were recorded after each spray.

The data was subjected to analysis of variance (ANOVA) using SPSS (version 16). Tukey's test was used to test the significance of difference between the treatment means.

RESULTS AND DISCUSSION

The effect of elicitor treatments on various growth characters of *B. juncea* and *B. napus* plants is presented in fig. 1. In both the cultivars, the 1st spray of

the various treatments showed no significant difference in plant height. After 2nd, 3rd and 4th spray, treatments containing combinations of elicitors, i.e., BTH (3 ppm) + SA (33 ppm) and BTH (7 ppm) + SA (17 ppm) resulted in significant increase in plant height followed by BTH (10 ppm), SA (50 ppm) and fungicide (blitox) as compared to control. Mean values for the plant height of *B. juncea* and *B. napus* plants were found in the range of 52.6-87.7 and 29.6-47.3 inches, respectively.

In *B. juncea*, BTH (3 ppm) + SA (33 ppm), BTH (7 ppm) + SA (17 ppm) and SA (50 ppm) showed significantly higher internodal distance after 1st spray. In *B. napus*, BTH (7 ppm) + SA (17 ppm) and BTH (3 ppm) + SA (33 ppm) exhibited significantly higher internodal distance after 1st spray. After 2nd, 3rd and 4th sprays, BTH (7 ppm) + SA (17 ppm) exhibited maximum internodal distance in both the cultivars. Mean values for the internodal distance of *B. juncea* and *B. napus* plants were found in the range of 2.8-6.1 and 1.3-5.0 inches, respectively.

In both the cultivars, the 1st spray of BTH (3 ppm) + SA (33 ppm) showed significantly maximum number of pods per plant. After 2nd, 3rd and 4th sprays, BTH (7 ppm) + SA (17 ppm) exhibited significantly maximum number of pods per plant followed by BTH (3 ppm) + SA (33 ppm), BTH (10 ppm), SA (50 ppm) and fungicide (blitox) as compared to control. The increase in growth parameters might be attributed to effect of elicitors on physiological processes of plants such as ion uptake, cell elongation, cell division, enzyme activation and protein synthesis. Our results are in agreement with those obtained by Shakirova *et al.* (2003) in wheat, Farouk *et al.* (2008) in cucumber, Amin *et al.* (2007) in onion and Farouk and Osman

Table 1. Effect of different treatments on total soluble protein content in leaves of *B. juncea* (cv. PBR-91) and *B. napus* (cv. GSC-6).

Elicitor/fungicide sprays	Total soluble protein content (mg/g fresh weight)			
	1 st spray	2 nd spray	3 rd spray	4 th spray
<i>B. juncea</i> (cv. PBR-91)				
BTH (10 ppm)	13.08 ± 0.90 ^{bcd}	13.82 ± 1.50 ^{bc}	15.32 ± 0.72 ^{cd}	16.56 ± 0.78 ^{cd}
SA (50 ppm)	12.51 ± 0.72 ^{bc}	12.75 ± 0.90 ^{abc}	14.51 ± 0.73 ^{bc}	15.44 ± 0.77 ^{bc}
BTH (3 ppm) + SA (33 ppm)	13.74 ± 0.71 ^{bc}	14.11 ± 0.80 ^{cd}	15.97 ± 0.62 ^{cd}	18.29 ± 0.83 ^d
BTH (7 ppm) + SA (17 ppm)	14.65 ± 0.97 ^c	15.26 ± 1.00 ^d	16.49 ± 0.52 ^d	17.18 ± 0.58 ^{cd}
Blitox (0.25%)	11.41 ± 1.00 ^{ab}	11.88 ± 0.47 ^b	13.25 ± 0.58 ^b	14.57 ± 0.62 ^b
Control (water-spray)	8.94 ± 0.86 ^a	10.49 ± 0.70 ^a	10.98 ± 0.86 ^a	11.96 ± 0.41 ^a
<i>B. napus</i> (cv. GSC-6)				
BTH (10 ppm)	11.37 ± 0.72 ^b	12.40 ± 1.10 ^{ab}	12.61 ± 0.90 ^{bc}	16.24 ± 0.55 ^{bc}
SA (50 ppm)	11.09 ± 0.61 ^b	11.80 ± 1.20 ^{ab}	12.20 ± 0.80 ^{bc}	15.66 ± 0.51 ^b
BTH (3 ppm) + SA (33 ppm)	12.49 ± 1.15 ^b	13.47 ± 1.20 ^{bc}	14.65 ± 0.76 ^{bc}	17.99 ± 0.83 ^c
BTH (7 ppm) + SA (17 ppm)	13.13 ± 0.44 ^b	14.63 ± 1.00 ^c	15.32 ± 1.00 ^c	17.92 ± 0.70 ^c
Blitox (0.25%)	10.59 ± 0.60 ^b	11.20 ± 1.20 ^{ab}	11.80 ± 0.61 ^{ab}	14.66 ± 0.70 ^b
Control (water-spray)	9.60 ± 0.84 ^a	9.89 ± 0.82 ^a	10.61 ± 0.32 ^a	12.55 ± 0.44 ^a

Data represent mean ± SD of three replications; Different letters indicate significant difference between treatments at p = 0.05 according to Tukey's test.

Table 2. Effect of different treatments on free amino acid content in leaves of *B. juncea* (cv. PBR-91) and *B. napus* (cv. GSC-6).

Elicitor/fungicide sprays	Free amino acid content (mg/g dry weight)			
	1 st spray	2 nd spray	3 rd spray	4 th spray
<i>B. juncea</i> (cv. PBR-91)				
BTH (10 ppm)	7.91 ± 0.40 ^b	5.51 ± 0.35 ^a	4.78 ± 0.25 ^b	4.12 ± 0.16 ^{ab}
SA (50 ppm)	7.59 ± 0.29 ^b	4.98 ± 0.16 ^a	4.52 ± 0.69 ^{ab}	3.93 ± 0.27 ^{ab}
BTH (3 ppm) + SA (33 ppm)	7.99 ± 0.15 ^b	6.60 ± 0.31 ^b	6.89 ± 0.46 ^c	5.81 ± 0.30 ^c
BTH (7 ppm) + SA (17 ppm)	9.88 ± 0.40 ^c	9.56 ± 0.39 ^c	9.06 ± 0.31 ^d	8.72 ± 0.41 ^d
Blitox (0.25%)	6.44 ± 0.58 ^a	4.92 ± 0.24 ^a	4.33 ± 0.63 ^{ab}	3.93 ± 0.09 ^b
Control (water-spray)	5.39 ± 0.52 ^a	4.83 ± 0.17 ^a	3.97 ± 0.45 ^a	3.05 ± 0.08 ^a
<i>B. napus</i> (cv. GSC-6)				
BTH (10 ppm)	17.63 ± 0.48 ^d	14.73 ± 0.81 ^c	11.01 ± 0.51 ^{cd}	8.56 ± 0.15 ^c
SA (50 ppm)	15.87 ± 0.35 ^c	10.60 ± 0.42 ^b	9.48 ± 0.41 ^{ab}	7.87 ± 0.12 ^c
BTH (3 ppm) + SA (33 ppm)	20.34 ± 0.51 ^e	18.41 ± 0.79 ^d	15.96 ± 0.59 ^e	12.79 ± 0.51 ^e
BTH (7 ppm) + SA (17 ppm)	18.37 ± 0.62 ^d	15.16 ± 0.57 ^c	11.95 ± 0.62 ^d	9.66 ± 0.58 ^d
Blitox (0.25%)	11.94 ± 0.39 ^b	10.36 ± 0.21 ^{bc}	8.38 ± 0.75 ^a	6.59 ± 0.28 ^b
Control (water-spray)	10.40 ± 0.55 ^a	8.59 ± 0.53 ^a	8.28 ± 0.43 ^a	5.44 ± 0.18 ^a

Data represent mean ± SD of three replications; Different letters indicate significant difference between treatments at p = 0.05 according to Tukey's test.

(2011) in bean who found that low doses of SA resulted in enhanced plant growth.

The total soluble protein content of leaves of both *B. juncea* and *B. napus* increased in all the treatments of both the cultivars after each spray (Table 1). It was observed that BTH (7 ppm) + SA (17 ppm) exhibited maximum total soluble protein content in both the cultivars after each spray. In *B. juncea*, this treatment showed 1.64-, 1.45-, 1.50- and 1.44-fold high total soluble protein content than control after 1st, 2nd, 3rd and 4th sprays, respectively. In *B. napus*, it showed 1.37-, 1.48-, 1.44- and 1.43-fold high total soluble protein content than control after 1st, 2nd, 3rd and 4th sprays, respectively. Increase in total soluble protein might be due to the metabolic reactions associated with disease resistance such as synthesis of specific proteins related to infection (Onifade and Agboola, 2003; Misra *et al.*, 2008). El- Khallal (2007) observed that jasmonic acid and SA significantly increased the total soluble protein content in tomato plants as compared to infected control plants.

Free amino acid content decreased in all the treatments of both the cultivars after each spray (Table 2). In *B. juncea*, BTH (7 ppm) + SA (17 ppm) exhibited maximum free amino acid content compared to other treatments after each spray. This treatment showed 1.83-, 1.98-, 2.28- and 2.86-fold high free amino acid content than control after 1st, 2nd, 3rd and 4th sprays, respectively. In *B. napus*, BTH (3 ppm) + SA (33 ppm) showed 1.95-, 2.14-, 1.93- and 2.35-fold high free

amino acid content than control after 1st, 2nd, 3rd and 4th sprays, respectively. El- Khallal (2007) observed higher free amino acid content in tomato plants treated with elicitors *viz.*, jasmonic acid and SA as compared to infected control plants. Decrease in free amino acid content might be due to their utilization by the pathogens. The fungus induces catabolic breakdown of amino acid and amides into nitrogen (Singh *et al.*, 2011).

Total sugars and reducing sugars content decreased in all the treatments of both the cultivars after each spray (Table 3 and 4). In *B. juncea*, 1st spray of elicitors and fungicide exhibited significantly higher total sugars content than control. Treatment containing BTH (3 ppm) + SA (33 ppm) showed 1.31-, 1.34- and 1.36-fold high total sugars content than control after 2nd, 3rd and 4th sprays, respectively. In *B. napus*, BTH (7 ppm) + SA (17 ppm) exhibited about 1.46-fold increase in total sugars content than control after 1st, 2nd and 3rd sprays, respectively. After 4th spray, the same treatment showed 1.61-fold high total sugars content than control.

In *B. juncea*, BTH (3 ppm) + SA (33 ppm) showed about 1.94-fold high content of reducing sugars than control after 1st and 2nd sprays. After 2nd and 3rd sprays, the same treatment exhibited 1.79- and 1.88-fold high reducing sugars content, respectively as compared to control. In *B. napus*, BTH (7 ppm) + SA (17 ppm) showed 1.88-, 1.83-, 2-, 2.16- and 2.42-fold high reducing sugars content, respectively as compared to

Table 3. Effect of different treatments on total sugars content in leaves of *B. juncea* (cv. PBR-91) and *B. napus* (cv. GSC-6).

Elicitor/fungicide sprays	Total sugars content (mg/g dry weight)			
	1 st spray	2 nd spray	3 rd spray	4 th spray
<i>B. juncea</i> (cv. PBR-91)				
BTH (10 ppm)	50.19 ± 2.39 ^b	42.07 ± 1.81 ^{bc}	40.76 ± 1.52 ^{ab}	38.01 ± 2.08 ^{ab}
SA (50 ppm)	54.69 ± 4.57 ^b	40.14 ± 2.39 ^{ab}	37.82 ± 1.95 ^{ab}	36.32 ± 2.47 ^a
BTH (3 ppm) + SA (33 ppm)	54.80 ± 3.48 ^b	46.98 ± 2.00 ^c	45.86 ± 3.50 ^b	44.17 ± 2.48 ^c
BTH (7 ppm) + SA (17 ppm)	53.84 ± 3.62 ^b	45.46 ± 2.88 ^{bc}	44.49 ± 2.35 ^b	43.02 ± 1.67 ^{bc}
Blitox (0.25%)	46.79 ± 3.37 ^b	40.65 ± 2.44 ^{ab}	39.65 ± 4.78 ^{ab}	35.77 ± 1.80 ^a
Control (water-spray)	37.29 ± 2.30 ^a	35.70 ± 1.55 ^a	34.27 ± 4.15 ^a	32.48 ± 2.00 ^a
<i>B. napus</i> (cv. GSC-6)				
BTH (10 ppm)	57.90 ± 4.34 ^b	52.62 ± 2.38 ^{bc}	48.52 ± 1.98 ^{bc}	45.35 ± 3.38 ^{bc}
SA (50 ppm)	53.25 ± 2.12 ^{ab}	50.44 ± 4.46 ^{abc}	47.42 ± 3.22 ^{bc}	44.07 ± 3.03 ^{bc}
BTH (3 ppm) + SA (33 ppm)	60.50 ± 4.53 ^{bc}	56.21 ± 2.93 ^c	51.14 ± 3.02 ^c	49.56 ± 1.07 ^c
BTH (7 ppm) + SA (17 ppm)	69.19 ± 4.71 ^c	59.93 ± 4.51 ^c	52.09 ± 1.97 ^c	50.91 ± 2.94 ^c
Blitox (0.25%)	51.05 ± 3.25 ^{ab}	44.90 ± 3.89 ^{ab}	43.79 ± 2.06 ^b	40.64 ± 3.59 ^b
Control (water-spray)	47.34 ± 2.02 ^a	40.91 ± 2.48 ^a	35.64 ± 2.61 ^a	31.58 ± 2.06 ^a

Data represent mean ± SD of three replications; Different letters indicate significant difference between treatments at $p = 0.05$ according to Tukey's test.

control. These results are supported by the findings of Jaypal and Mahadevan (1968), who reported post-infectious decrease in sugar levels caused by rapid hydrolysis of sugars during pathogenesis through

enzymes secreted by the pathogen. The invading pathogens may utilize the sugar leading to decrease in its content. El-Khallal (2007) observed higher sugar content in tomato plants treated with jasmonic acid and

Table 4. Effect of different treatments on reducing sugars content in leaves of *B. juncea* (cv. PBR-91) and *B. napus* (cv. GSC-6).

Elicitor/fungicide sprays	Reducing sugars content (mg/g dry weight)			
	1 st spray	2 nd spray	3 rd spray	4 th spray
<i>B. juncea</i> (cv. PBR-91)				
BTH (10 ppm)	1.35 ± 0.13 ^{bc}	1.33 ± 0.17 ^{cd}	1.00 ± 0.09 ^{bc}	0.90 ± 0.12 ^{bc}
SA (50 ppm)	1.28 ± 0.11 ^b	1.17 ± 0.06 ^{bc}	0.83 ± 0.10 ^{ab}	0.80 ± 0.08 ^{ab}
BTH (3 ppm) + SA (33 ppm)	1.62 ± 0.08 ^c	1.49 ± 0.10 ^d	1.13 ± 0.13 ^c	1.09 ± 0.07 ^c
BTH (7 ppm) + SA (17 ppm)	1.55 ± 0.11 ^{bc}	1.39 ± 0.12 ^{cd}	1.00 ± 0.07 ^{bc}	0.99 ± 0.14 ^{bc}
Blitox (0.25%)	0.98 ± 0.11 ^a	0.96 ± 0.07 ^{ab}	0.77 ± 0.03 ^{ab}	0.71 ± 0.07 ^{ab}
Control (water-spray)	0.83 ± 0.06 ^a	0.77 ± 0.07 ^a	0.63 ± 0.10 ^a	0.58 ± 0.10 ^a
<i>B. napus</i> (cv. GSC-6)				
BTH (10 ppm)	1.55 ± 0.09 ^{cd}	1.46 ± 0.06 ^c	1.28 ± 0.17 ^c	0.96 ± 0.09 ^{bc}
SA (50 ppm)	1.31 ± 0.07 ^{bc}	1.23 ± 0.15 ^b	1.11 ± 0.16 ^{bc}	0.91 ± 0.11 ^{bc}
BTH (3 ppm) + SA (33 ppm)	1.66 ± 0.12 ^d	1.57 ± 0.07 ^c	1.34 ± 0.15 ^c	1.14 ± 0.15 ^{cd}
BTH (7 ppm) + SA (17 ppm)	1.79 ± 0.10 ^d	1.62 ± 0.05 ^c	1.47 ± 0.21 ^c	1.33 ± 0.18 ^d
Blitox (0.25%)	1.09 ± 0.10 ^{ab}	0.94 ± 0.08 ^a	0.84 ± 0.11 ^{ab}	0.68 ± 0.06 ^d
Control (water-spray)	0.98 ± 0.10 ^a	0.81 ± 0.04 ^a	0.68 ± 0.05 ^a	0.55 ± 0.06 ^a

Data represent mean ± SD of three replications; Different letters indicate significant difference between treatments at $p = 0.05$ according to Tukey's test.

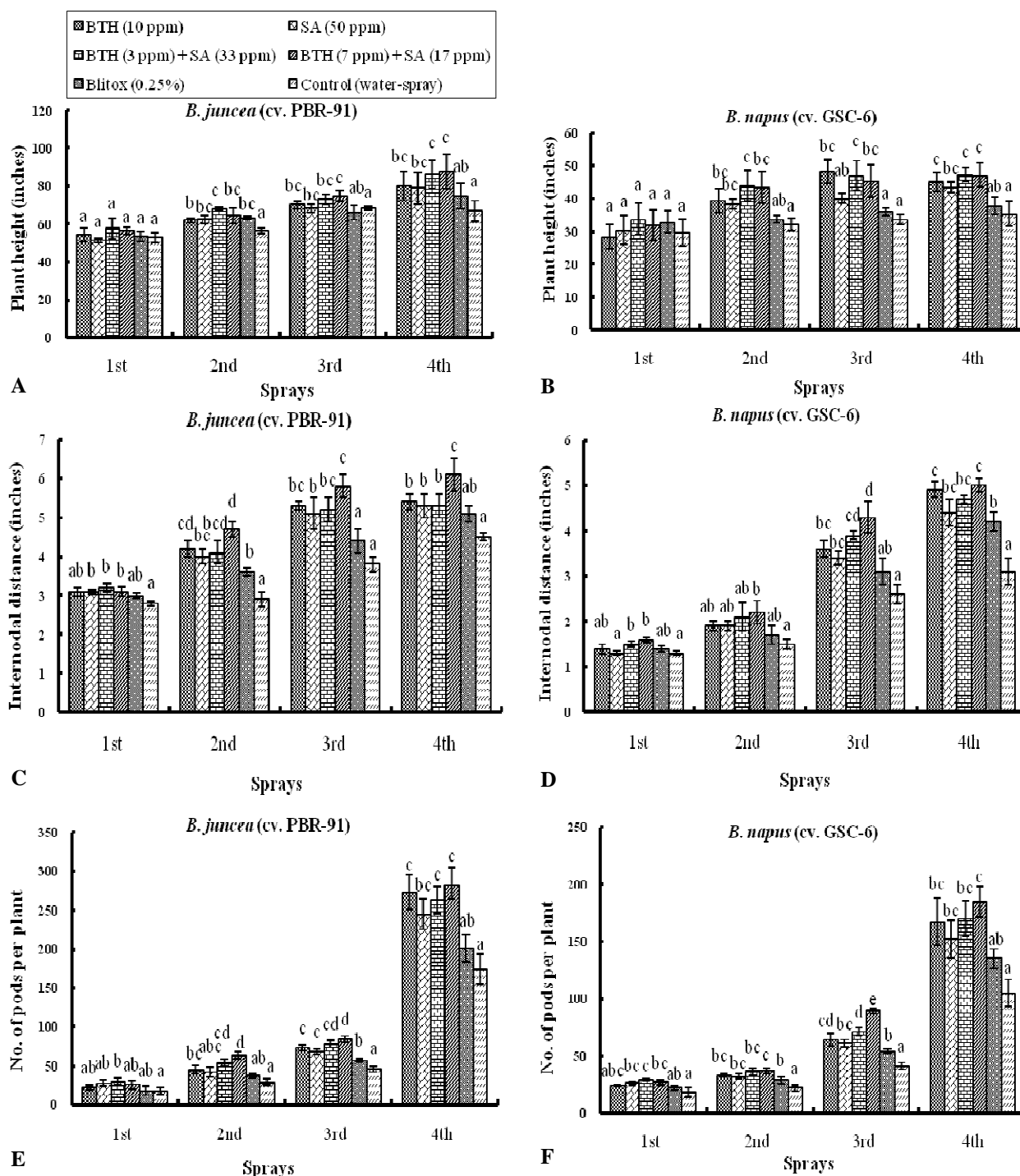


Fig.1. Effect of different treatments on plant height of *B. juncea* (A) and *B. napus* (B); internodal distance of *B. juncea* (C) and *B. napus* (D); number of plants per plant of *B. juncea* (E) and *B. napus* (F). Data represent mean \pm SD of three replicates. Different letters indicate significant difference between treatments at $p = 0.05$, according to Tukey's test.

SA than infected control plants.

Conclusion

The content of leaves of total soluble protein of both *B. juncea* and *B.napus* increased, whereas free amino acids, total sugars and reducing sugars decreased after each spray. Plants treated with elicitors resulted in higher contents of total soluble protein, free amino

acids, total sugars and reducing sugars than water treated control plants. Elicitors also affected the physiomorphological processes related to growth and development. Among all the treatments, the combinations of elicitors were highly effective. Fungicide also exhibited higher content of metabolites than control, but it was less than elicitor treatments. Thus, exogenous application of combinations of

elicitors might improve growth and disease resistance of *B. juncea* and *B. napus* cultivars.

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